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„Plasma applications for smart and sustainable agriculture“
PLASMAS FOR PLANT AND FOOD PROCESSING
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VYTAUTAS MAGNUS UNIVERSITY
MCMXXII

Do plasma effects on seed germination predict effects on plant growth and productivity?

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Historically, the first reports on the application of plasma in plant biology were published in 2000 and described the effects on seed germination induced by plasma treatment. Pionering reports:

- **Dubinov et al., 2000**: oat (*Avena sativa*) and barley (*Hordeum vulgare*) seeds were treated with air glow discharge for several minutes. The continuous mode stimulated seed germination more effectively than the pulsed mode but no changes in early growth of the seedlings were observed.
- **Volin et al., 2000**: longer treatments (2–20 min) of low-pressure radio frequency (RF) rotating plasma in fluorocarbon or nitrogen or carbon-containing compound atmosphere were applied for the treatment of barley (*Hordeum vulgare*), radish (*Raphanus sativus*), pea (*Pisum sativum*), soybean (*Glycine max*), corn (Zea mays), and bean (*Phaseolus vulgaris*) seeds. Strong negative effects on germination was observed in the majority of cases.
- **Živkovic, S. et al., 2004:** reported stimulation of empress tree (*Paulownia tomentosa*) seed germination by plasma treatment and suggested three different physical mechanisms: etching, surface functionalization, and deposition of small bioactive molecules.
- **Meiqiang et al. , 2005:** tomato seeds were treated with magnetized plasma (arc discharge combined with magnetic field) and no effects on germination *in vitro* was observed. However, seedling emergence in pots was enhanced, and some of the treatment protocols resulted in increased activity of enzymes in seedling tissues (peroxidase in hypocotyls and dehydrogenase in roots), as well as an increase in the number of fruits and fruit biomass per plant.
- **Šerá et al., 2008:** strong stimulation of Lamb's Quarters (*Chenopodium album* agg.) seed germination after using low-pressure microwave plasma treatment was explained by cracks found on the seed surface (electron microscope scanning), where water could better penetrate seeds.

Since then, seed germination is regarded as the main criterion of plasma treatment effects.
Intuitively, it is expected that treatments stimulating germination exert positive effects on other plant properties.
Are such expectations evidence-based?

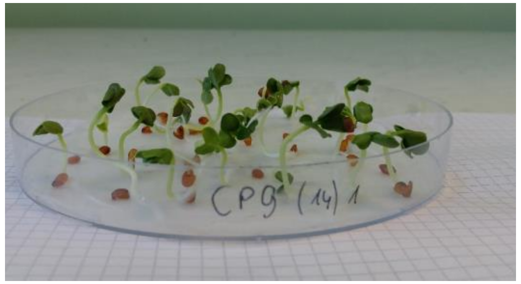
The mechanisms of effects of pre-sowing seed treatment with cold plasma on seed germination and plant growth

Statements coming from the majority of publications dealing with plasma effects:

CP treatments increase seed quality due to:

- (1) microbial decontamination of seeds;
- (2) stimulation of seed germination.

The majority of studies were limited by effects on seed surface (wettability), seed germination and early seedling growth



Positive effects on seed decontamination, germination and seedling growth are regarded as evidence for the usefulness of plasma application in agriculture

Germination - the emergence of a new plant from a seed. It starts with water uptake by the dry seed and is complete when the elongating radicle traverses the seed coat.

Emergence of the embryo, usually a radicle (embryonic root) is called *sensu stricto* germination.
Emergence of seedling in the substrate includes a later stage of seedling growth
(different from a *sensu stricto germination)*

Factors affecting germination

- **Water -** germination occurs when the seed absorbs enough water to rupture the seed coat.
- Rehydration of tissues - dilution of inhibitors

Hydration of a seed, which is called imbibition (*imbibere* in Latin means *to drink*), is an essential step for seed germination.

- **Oxygen**
  Aerobic respiration
- **Temperature**
  Enzyme controlled processes
- Some seeds may require exposure to light or high temperatures (fire).

Germination leads to the elongation of the embryonic axis from a seed, allowing subsequent seedling emergence. Elongation of emerged roots and seedling development are referred to as post-germination events

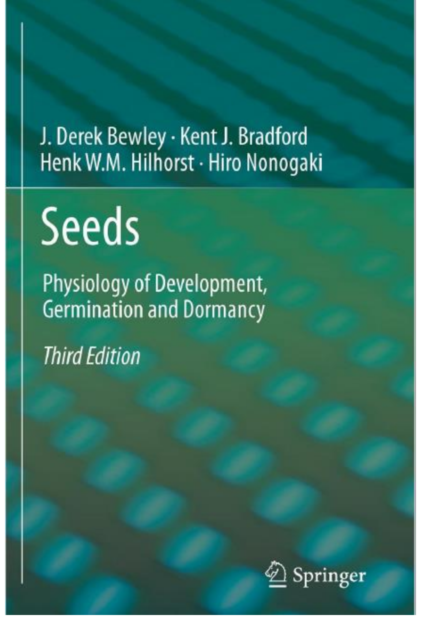


Fig. 4.1 Time course of water uptake and some important changes associated with germination and early seedling growth.
Initial absorption of water, imbibition in Phase I, is primarily a physical process; physiological activities may commence within minutes of a cell becoming hydrated, well before all seed tissues become fully imbibed.
During Phase II seed water content is fairly constant and metabolic activities increase with substantial transcription of new genes. Radicle emergence through the surrounding structures at the end of this Phase marks the completion of germination.
In Phase III there is further uptake of water as the young seedling becomes established, utilizing the major stored reserves.

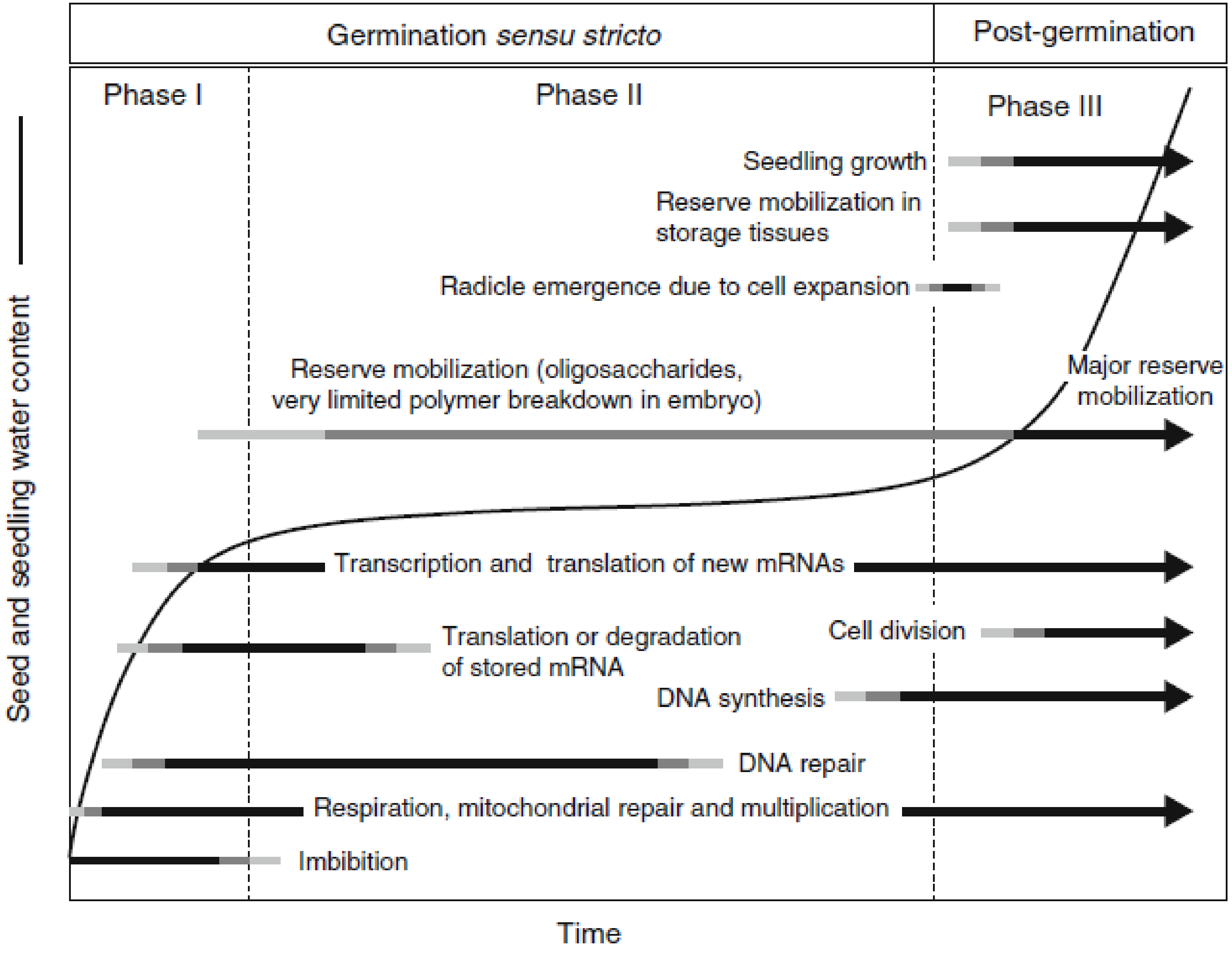


Fig. 4.1 Time course of water uptake and some important changes associated with germination and early seedling growth. Initial absorption of water, imbibition in Phase I, is primarily a physical

How seed treatment with plasma stimulates seed germination?

Popular explanation: Plasma stimulates seed germination due to chemical modifications on seed surface leading to increased wettability and water absorption

Suggested by E. Bormashenko et al. Cold Radiofrequency Plasma Treatment Modifies Wettability and Germination Speed of Plant Seeds. Sci. Rep. 2012, 2, 741.

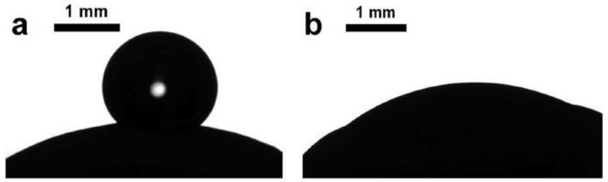
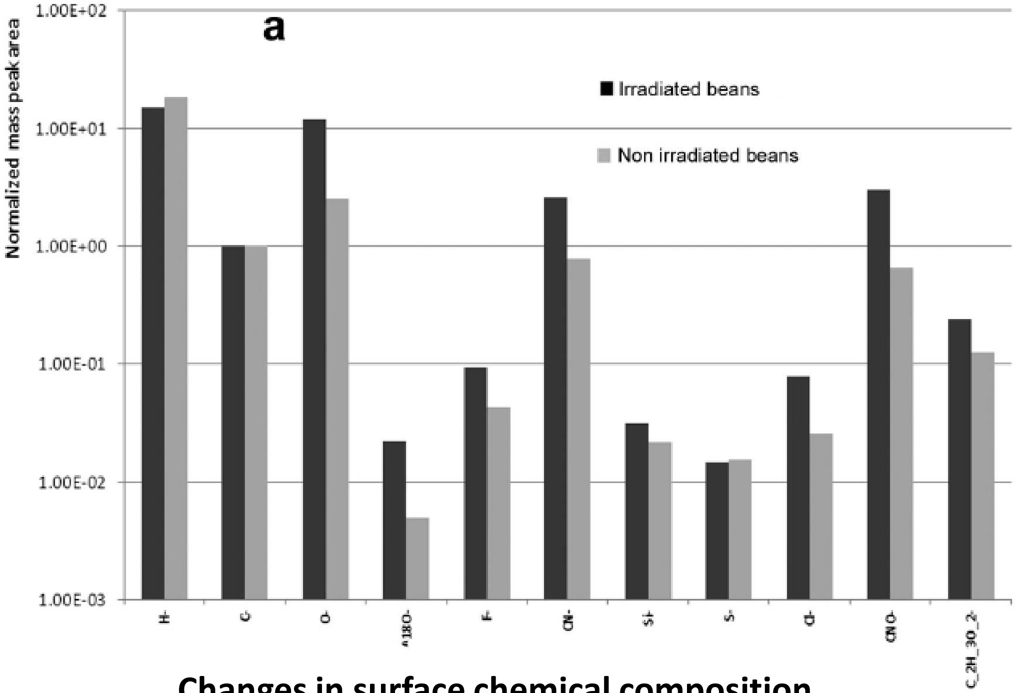


Figure 2 | Water droplet deposited on untreated (a) and cold plasma treated (b) lentil seed.

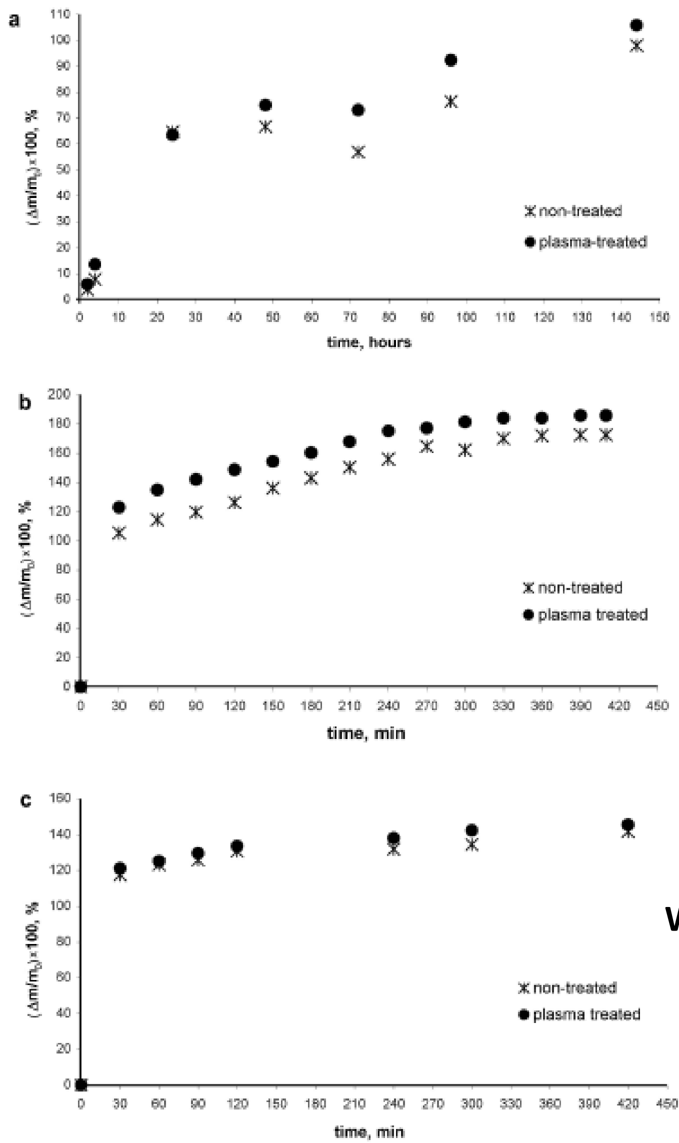


Changes in surface chemical composition

TOF-SIMS MS analysis

Water absorption

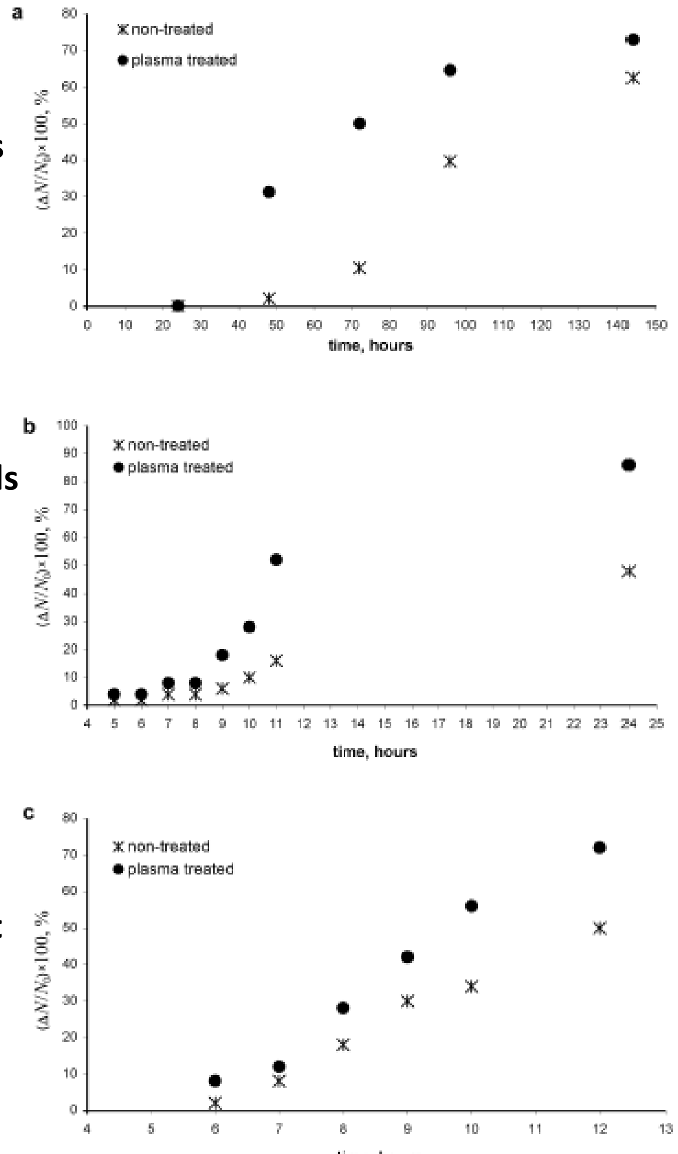
Germination



Beans

Lentils

Wheat

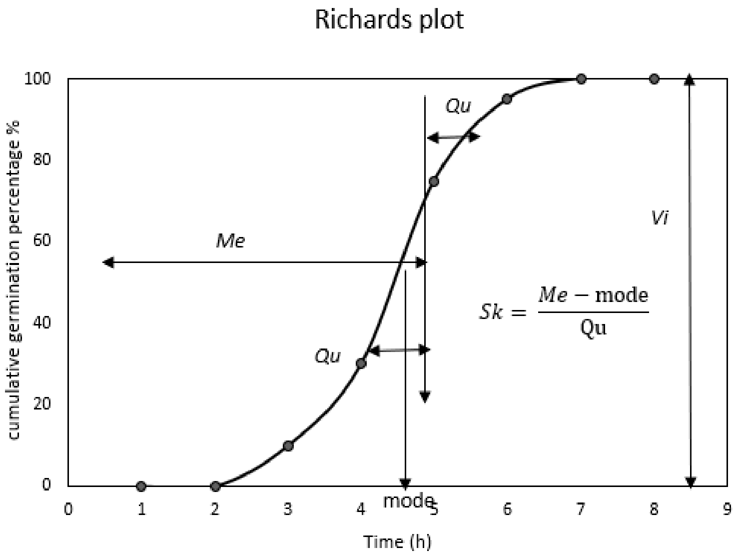


Three cultivars of pea (*Pisum sativum*): ‘Ieva’ (A), ‘Jura’ (B), ‘Kiblukai’ (C)

‚Ieva’	Control	CP2	CP7	EMF15	Vacuum
V_i (%)	96.7±0.0	96.7±1.9	97.8±1.1	91.1±3.3	85.6±11.3
M_e (hours)	79.7±0.2	87.0±5.6	78.6±5.7	**118.4±1.6***	**99.1±6.4***

‘Jura’	Control	CP2	CP7	EMF15	Vacuum
V_i (%)	95.6±2.9	93.3±5.1	95.6±1.1	100±0.0	97.8±2.2
M_e (hours)	115.2±14.2	121.6±12.2	115.3±9.5	104.5±4.9	93.9±5.8

‚Kiblukai’	Control	CP2	CP7	EMF15	Vacuum
V_i (%)	98.9±1.1	100±0.0	95.6±4.4	97.8±1.1	100±0.0
M_e (hours)	65.5±0.5	70.6±9.0	**59.8±0.8* -8.7%**	**68.2±0.7* +3.96%**	67.3±2.6

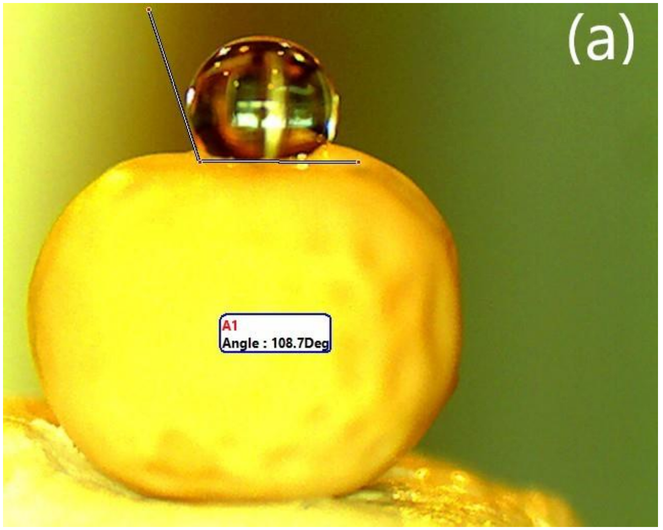


Vi (%) – the final germination percentage indicating seed viability,
Me (hours) – the median germination time ($t_{50\%}$), indicating the germination halftime or 1/germination rate.

Changes in seed surface wettability (water contact angle)

Pea cultivar	Control	CP2	CP7	EMF15	Vacuum
‚Ieva'	101.2±2.8	82.6±2.4*	75.7±7.4*	103.3±1.8	95.7±1.7
‚Jura'	97.3±3.2	83.8±0.9*	83.9±1.5*	101.1±1.6	96.3±3.6
‚Kiblukai'	109±2.4	102.9±3.0*	101.8±1.8*	107.9±2.6	114.8±2.0
T. baccata	105.5±2.4		95.7±1.5*		

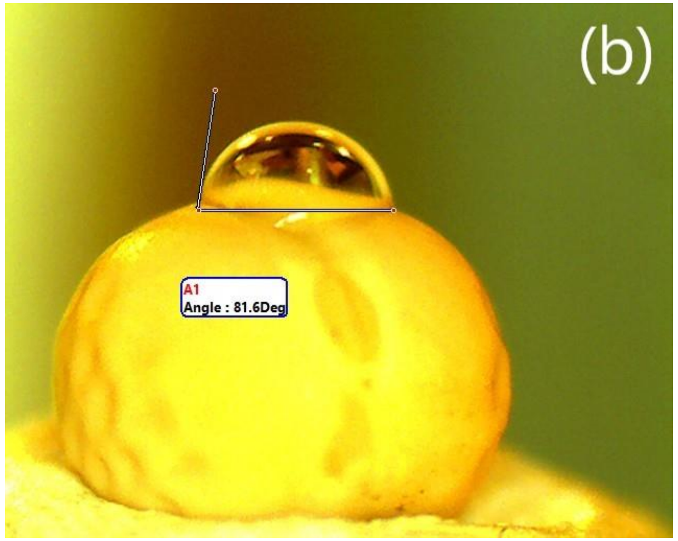
n=6 seeds for each group, colors indicate effects on germination



CP treatments significantly increase wettability of seed coat in all seeds;

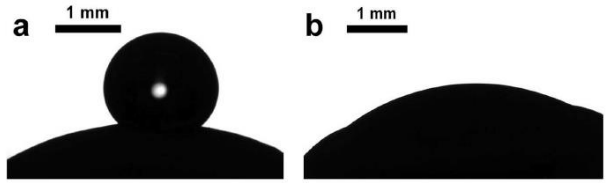
EMF and vacuum treatments – do not change seed surface wettability;

Increase in wettability by CP is observed in seeds with not affected germination ('Ieva', 'Jura'; 'Kiblukai', (CP2));
Positive effect of EMF on germination was observed for 'Kiblukai' seeds with unchanged wettability



Increased seed wettability in the case of negative effects on germination:
A. Los, D. Ziuzina, D. Boehm, P. J. Cullen, P. Bourke. Investigation of mechanisms involved in germination enhancement of wheat (*Triticum aestivum*) by cold plasma: Effects on seed surface chemistry and characteristics. *Plasma Process Polym.* 2019;e1800148.

Qestion for believers to “increased seed wettability” explanation



If “increased seed coat surface chemical composition, structure and wettability” can also have impact on plant growth for the entire vegetation period and harvest?

Recently, experimental evidences accumulated that CP treatments stimulate germination by activation of numerous biochemical and molecular processes inside dry seeds

Finding	References	Plant species	Implication
Increased number of paramagnetic centers (EPR signal) in seeds	• Paužaitė et al. *Plasma Process. Polym*. **2018**, 15, 1–11. • Koga et al. *Jpn. J. Appl. Phys*. **2020**, *59*, SHHF01. • Attri et al. *Sci. Rep*. **2021**, *11*, 2539.	• Norway spruce • Radish • Radish	CP treatment increases production of stable organic radicals in seeds, indicating interaction of seed pigments with ROS from plasma or internally produced ROS
Increased H_2O_2 amount in dry and in germinating seeds	• Rahman et al. *Sci. Rep*. **2018**, *8*, 10498 • Paužaitė et al. *Plasma Process. Polym*. **2018**, 15, 1–11.	• Wheat • Norway spruce	CP induces internal ROS production
Shift in the balance of phytohormones involved in the control of germination	• Mildažienė et al. *Sci. Rep*. **2019**, *9*, 6437. • Zukiene et al. *Appl. Phys. Express* **2019**, 126003. • Degutytė-Fomins et al. *Jpn. J. Appl. Phys*. **2020**, *59*, SH1001. • Mildažienė et al. *Plasma Proc. Polym*. **2020**, *18*, 2000160. • Attri et al. *Sci. Rep*. 2021, *11*, 2539.	• Sunflower • Radish • Radish • Red clover • Radish	CP stimulates germination inducing drop in GA/ABA and change in the amounts of other plant hormones
Changes in DNA methylation	• Suriyasak et al. *ACS Agricultural. Sci.Techology*. **2021**, *1*, 5-10.	• Rice	CP induces changes in gene expression through changes in DNA methylation.
Changes in the expression of genes and expression of proteins	• Ji et al. *Arch. Biochem. Biophys*. **2016**, *605*, 117–128, • Rahman et al. *Sci. Rep*. **2018**, *8*, 10498 • Suriyasak et al. *ACS Agricultural. Sci.Techology*. **2021**, *1*, 5-10. Personal observations (unpublished)	• Spinach • Wheat • Rice • Sunflower	CP induces changes in expression of enzymes involved in mobilisation of resources for germination and repair of cellular structures

Summary of the experimentally proven effects of seed treatment with CP on seedlings and plants

Finding	References	Plant species	Comment
Effects on early seedling growth	• Numerous publications (cited in reviews 2020-2021)	• Many species	Extent of effect and optimal dose is species (cultivar, genotype) dependent
Changes in DNA methylation	• Zhang et al. *Sci. Rep.* **2017**, *7*, 41917.	• Soybean	Changes in gene expression through DNA methylation.
Changes in protein expression, including photosynthetic system, stress response, secondary metabolism	• Zhang et al. *Sci. Rep.* **2017**, *7*, 41917. • Mildažienė et al, *Sci. Rep.* **2019**, *9*, 6437. • Iranbakhsh et al. *Plasma Chem. Plasma Proc.* ***2020***, 40, 527–537 • Ghaemi et al. *J. Theor. Appl. Phys*, **2020**, 14, 323–328.	• Sunflower • Sunflower • Industrial hemp • *S. nemorosa*	CP induces significant changes in the amounts of proteins in growing plants
Effects on enzyme activities	• Numerous publications (cited in reviews 2020-2021)	•Many species	CP induces significant changes in plant metabolism
Stimulation of photosynthesis	• Saberi et al., *Sci. Rep.* **2018**, *8, 11655* • Zukiene et al. *Appl. Phys. Express* **2019**, 126003.	• Wheat • Sunflower	Upregulated photosynthesis results in better plant growth
Stimulation of secondary metabolism	• Mildažienė et al. *Plasma Process. Polym.* **2018**, *14*, 1700059. • Mildaziene et al. *J. Phys. D: Appl. Phys.* **2020**, *53*, 26. • Ivankov et al., *Appl. Sci*, **2020**, *10*, 8519.	• E. purpurea • Red clover • Industrial hemp	Stimulated SM leads to better plant establishment, fitness and stress resistance
Changes in plant communication with microorganisms including stimulation of N-fixation	• Tamošiūnė et al. *Front. Plant Sci.* **2020**, *11, 568924.* • Tamošiūnė et al. *Appl.* Phys. Express **2020**, *13*, 076001 • Pérez-Pizá et al. *Sci. Rep.* **2020**, *10*, 4917. • Mildažienė et al. *Plasma Proc. Polym.* **2020**, *18*, 2000160	•Sunflower •*A. thaliana* •Soybean •Red clover	CP effects "beyond plants" are important for improved agricultural performance
Enhanced growth for the entire vegetation and production yield (in field)	• Yin et al. Plasma Sci. Technol. 2005, 7, 3143–3147. • Zhou et al. Agricultural Science, 2 (1), 23–27. • Jiang et al. PLoS ONE 2014, 9, e97753 • Mildaziene et al. *J. Phys. D: Appl. Phys.* **2020**, *53*, 26 • Ivankov et al., *Appl. Sci*, **2020**, *10*, 8519.	•Tomato •Tomato •Wheat •Red clover •Industrial hemp	Persistence of CP effects implies that CP induces complex mechanisms of plant adaptation that can be exploited for Plasma in agriculture goals

What effects on agro-production yield (harvest) can be achieved by seed processing (without use of chemical fertilizers)?

- **Red clover:** biomass production increased up to 49% /grown in the field for 5 months/
- **Buckwheat:** strong positive effect on biomass (up to 97%) and on the seed yield (up to 85%) /grown in the field for 4 months/
- **Industrial hemp:** In female 'Futura 75' plants, EMF treatment induced positive changes in weight of the above ground part (66%) and number of inflorescences (70%). CP 5 min. treatment decreased the average weight of female plants by 27% but increased the weight of male plants 1.4-fold. /grown in the field for 4 months/
- Effects on growth of common buckwheat and industrial hemp in 2020 were qualitatively **reproduced** using different CP and EMF equipment. DBD plasma stimulated growth of 'Santica 27' cultivar by 30%, but inibited growth of 'Futura 75' cultivar plants...

Dr. Laima Degutytė-Fomins,
Dr. Giedre Paužaitė Maskeliūnienė,
Dr. Danuta Romanovska

Another explanation: breaking dormancy

For artificial dormancy breaking environmental dormancy breaking agents are used:

Temperature:
- Alternating temperatures (cold/warm cycles);
- Chilling (cold stratification);
- Warming (warm stratification);

Light:
- Alternating light (light/dark cycles);
- Single doses of light;
- Laser light

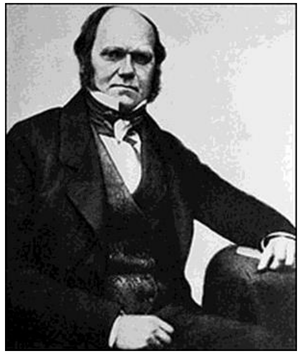
Chemicals:
- Smoke (fire, NO, butenolide)
- Inorganic;
- Organic (including allelochemicals).

Scarification:
- Mechanical;
- Chemical
- Enzymatic
- Percussion?

Ultrasound

High atmospheric pressure/Vacuum

Radiation:
- infrared or gas plasma (glow discharge, etc.) radiation,
- Low temperature plasma
- radio frequencies and ultrahigh frequency (microwave) electromagnetic fields,

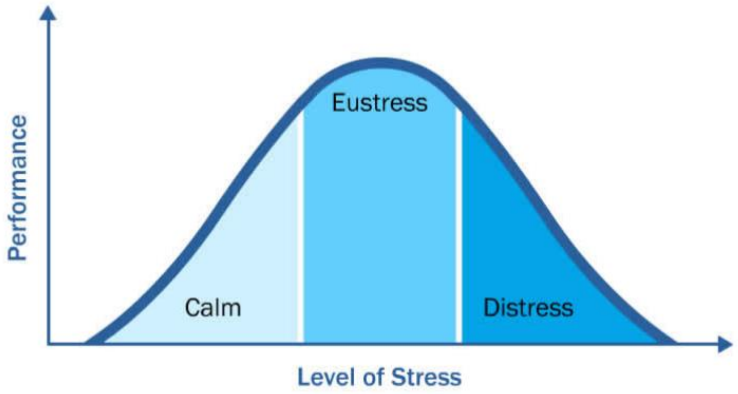


Charles Darwin was the first scientist who described positive effects of the pre-sowing seed treatment with salt solutions http://www.npr.org/templates/story/story.php?storyId=6105541

The Encyclopedia of Seeds– Science, Technology and Uses
J. Derek Bewley,Michael Black,Peter Halmer, eds, CABI, 2006

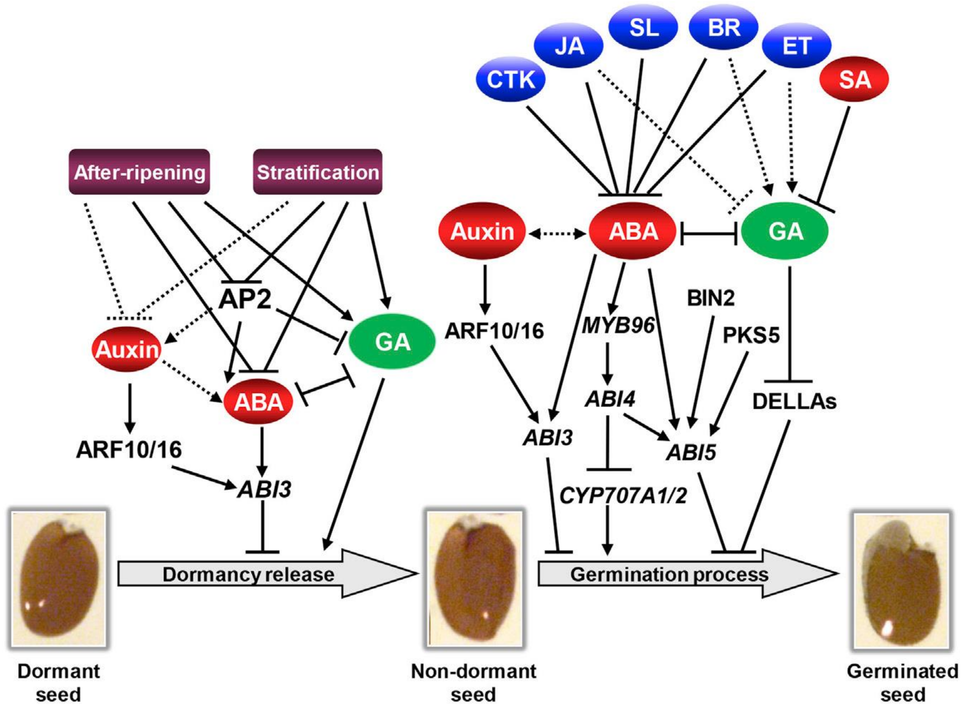
Carol C. Baskin,Jerry M. Baskin. **Seeds– Ecology, Biogeography, and, Evolution of Dormancy and Germination, Elsievier, 2014 , 2nd ed.**

Eustress or distress effects are strongly dependent on species and dose



The main regulators of dormancy:
abscisic acid ABA which counteracts gibberelins GA: ABA/GA duo

Dormant seeds have high ABA/GA ratio; dormancy breaking agents reduce ABA/GA ratio.



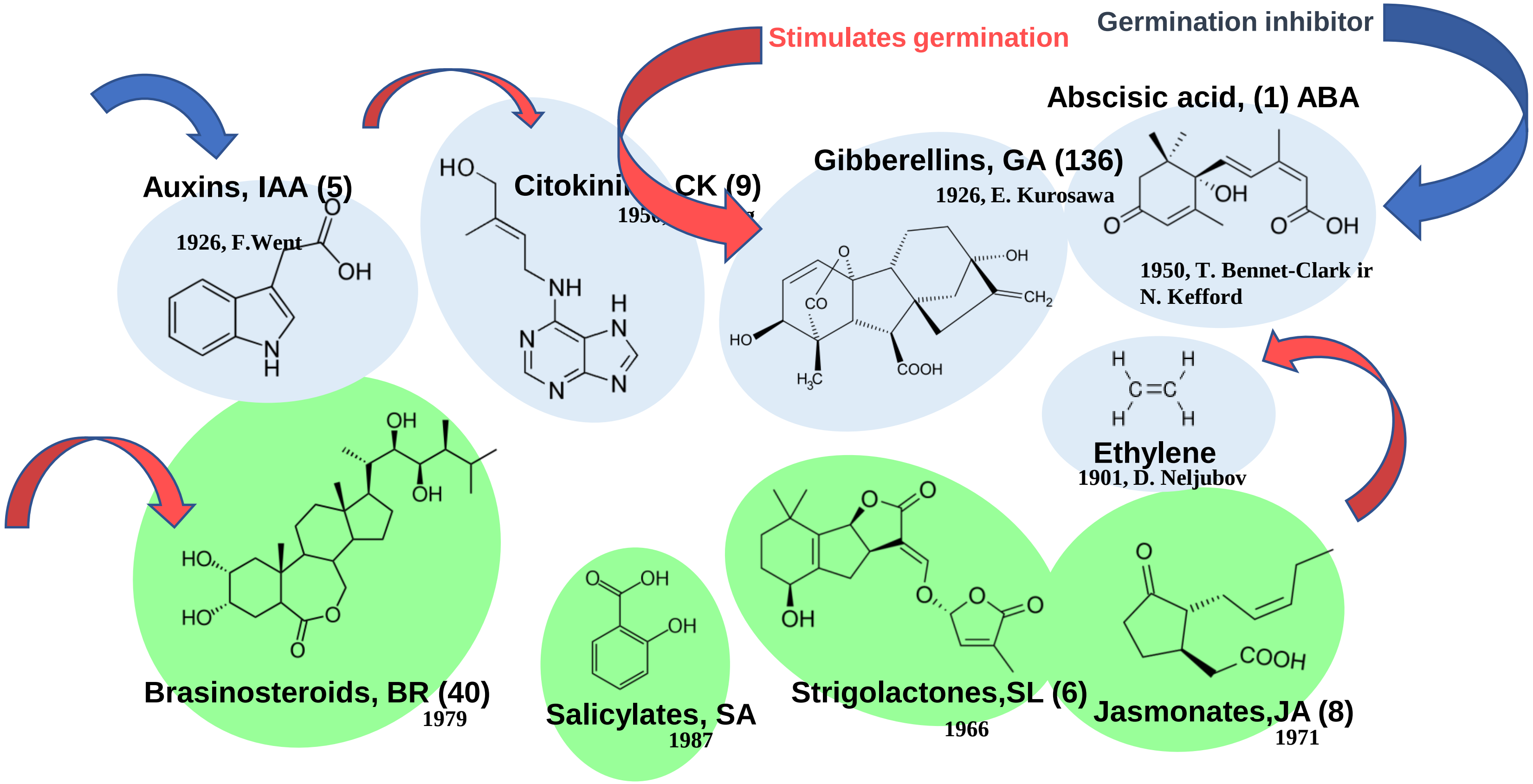
Shu K., Liu X.-d., Xie Q., and He Z.-h. (2016). Two Faces of One Seed: Hormonal Regulation of Dormancy and Germination. Mol. Plant. 9, 34–45.

Phytohormones act in concerted mutually interactive networks and modulate activities of other hormones

80% of plants belong to physiological type of seed dormancy: phytohormone dependent

Plant hormones (phytohormones)

a group of naturally occurring low-abundance organic compounds regulating all processes of plant life



Green colour – more recently found; the number of natural derivatives is indicated in parenthesis

Ivankov, A.; Zukiene, R.; Nauciene, Z. et al. The Effects of Red Clover Seed Treatment with Cold Plasma and Electromagnetic Field on Germination and Seedling Growth Are Dependent on Seed Color. Appl. Sci. 2021, 11, 4676.

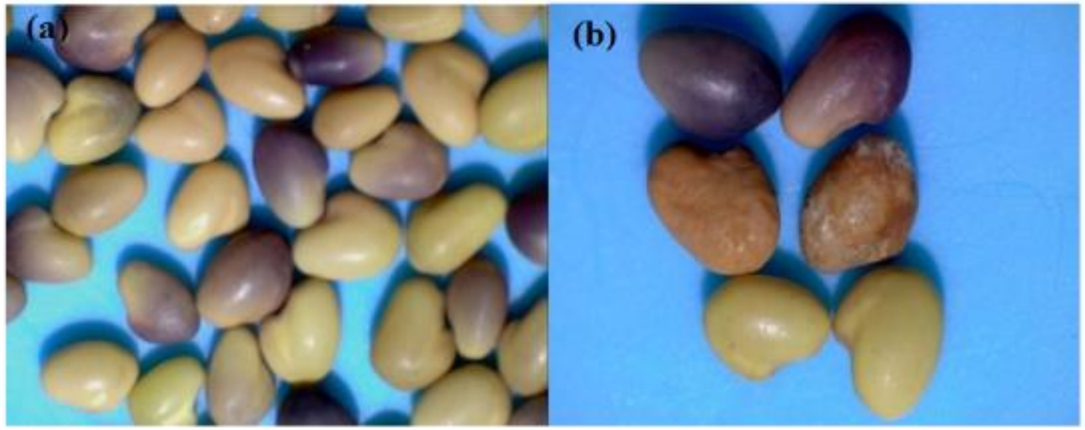


Figure 1. Variation of colors in a natural red clover seed lot (**a**); seeds of yellow, brown, and dark purple colors selected for the study (**b**).

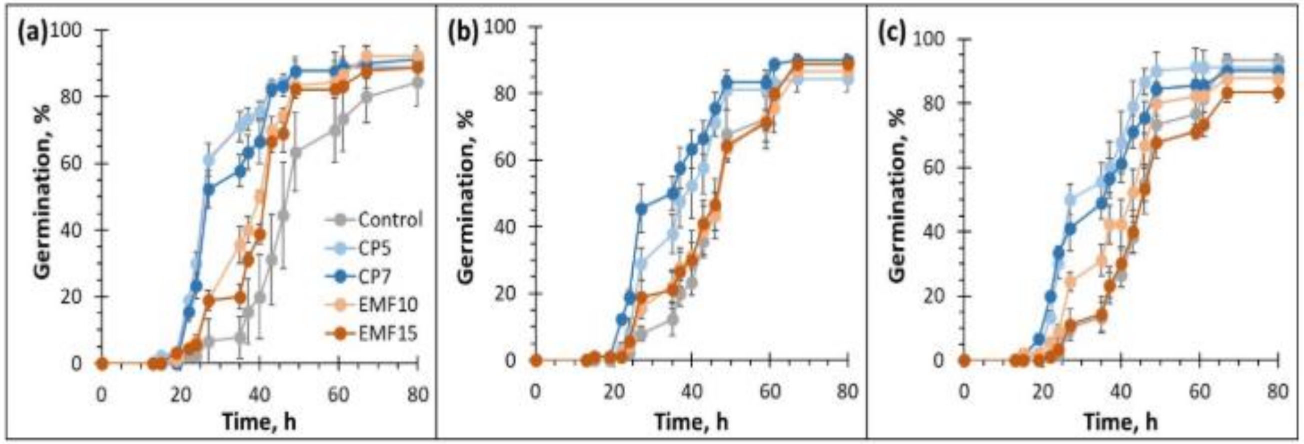


Figure 2. Germination dynamics of control and treated seeds of red clover—yellow (**a**), brown (**b**), and dark purple (**c**) seeds. Mean values of three replicates ± standard error are presented. The number of seeds in each replicate was 50 (n = 3).

Table 1. Indices of germination kinetics of control and treated red clover seeds of different color.

Seed Color	Germination Indices	Control	CP5	CP7	EMF10	EMF15
Yellow	V_i, %	84.4 ± 7.3	88.9 ± 6.1	91.1 ± 2.9	92.2 ± 2.9	89.0 ± 1.1
	M_e, h	44.4 ± 2.1	26.0 ± 1.5 *	30.2 ± 1.3 *	37.6 ± 0.9 *	39.5 ± 0.6 *
	Qu, h	5.1 ± 1.0	3.5 ± 1.3	6.6 ± 0.4	6.3 ± 0.4	5.3 ± 0.5
Brown	V_i, %	90.0 ± 1.9	84.4 ± 4.0	90.0 ± 1.9	86.7 ± 3.3	88.9 ± 2.9
	M_e, h	45.8 ± 2.0	35.9 ± 2.10 *	34.1 ± 0.8 *	44.6 ± 2.4	44.1 ± 1.7
	Qu, h	6.9 ± 1.1	6,9 ± 0.2	7.5 ± 1.20	8.9 ± 0.8	8.5 ± 0.7
Dark purple	V_i, %	93.3 ± 0.0	91.1 ± 4.0	90.0 ± 1.9	87.8 ± 4.8	88.9 ± 6.7
	M_e, h	43.8 ± 0.1	30.3 ± 3.9 *	31.9 ± 1.1 *	38.7 ± 0.8 *	42.8 ± 1.6
	Qu, h	6.3 ± 0.7	4.9 ± 1.6	8.5 ± 0.4 *	7.5 ± 0.5	6.5 ± 0.9

V_i, the final germination percentage; M_e, the median germination time; Q_u, the quartile deviation; results are presented as mean values ± standard errors; * significantly different from the control group ($p \leq 0.05$).

2023 experiment with dr. Bozena Sera (Comenius university in Bratislava) and prof. Radomira Vankova (Institute of Experimental Botany, Czech Academy of Sciences)

Comparison of phytohormone amounts (pmol/g FW) in yellow and dark purple seeds of red clover cv. 'Arimaiciai'

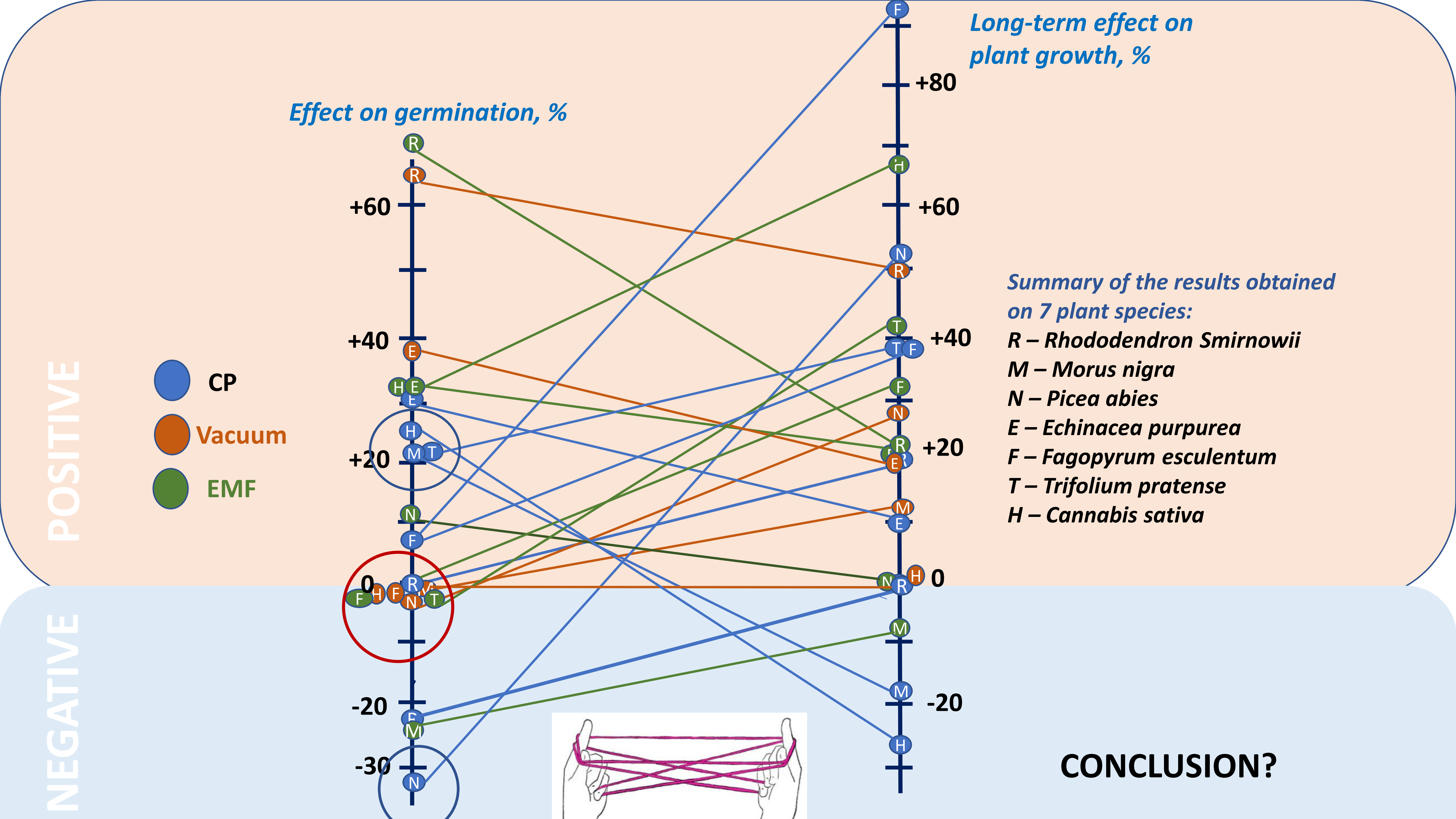
*significant effect of treatment
significant difference between yellow and dark purple seeds

Unpublished results removed

The effects on seed phytohormones matters

- Changes induced in the amounts/balance of seed phytohormones can exert “imprint” on amounts of phytohormones in the growing seedling/plant
- Phytohormones control all processes in plants, therefore their changes potentially can have systematic impact on plant behavior and performance
- How to understand the impact of phytohormonal shift – that is a question…

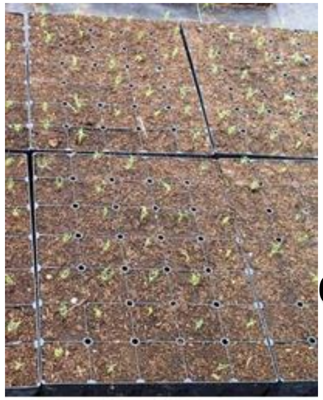
How effects of seed treatments on germination are related to effects on plant growth on the longer time scale (e.g. vegetation season)?



Effect on germination, %
Long-term effect on plant growth, %
POSITIVE
NEGATIVE
CP
Vacuum
EMF
+80
+60
+40
+20
0
-20
-30
Summary of the results obtained on 7 plant species:
R – Rhododendron Smirnowii
M – Morus nigra
N – Picea abies
E – Echinacea purpurea
F – Fagopyrum esculentum
T – Trifolium pratense
H – Cannabis sativa
CONCLUSION?

The observation of seed treatment effects on Norway spruce growth for 9 years:

2015-2023



cassetes

2015

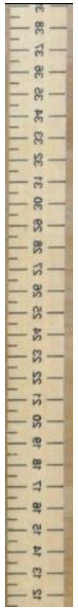
Outdoors

2016

pots

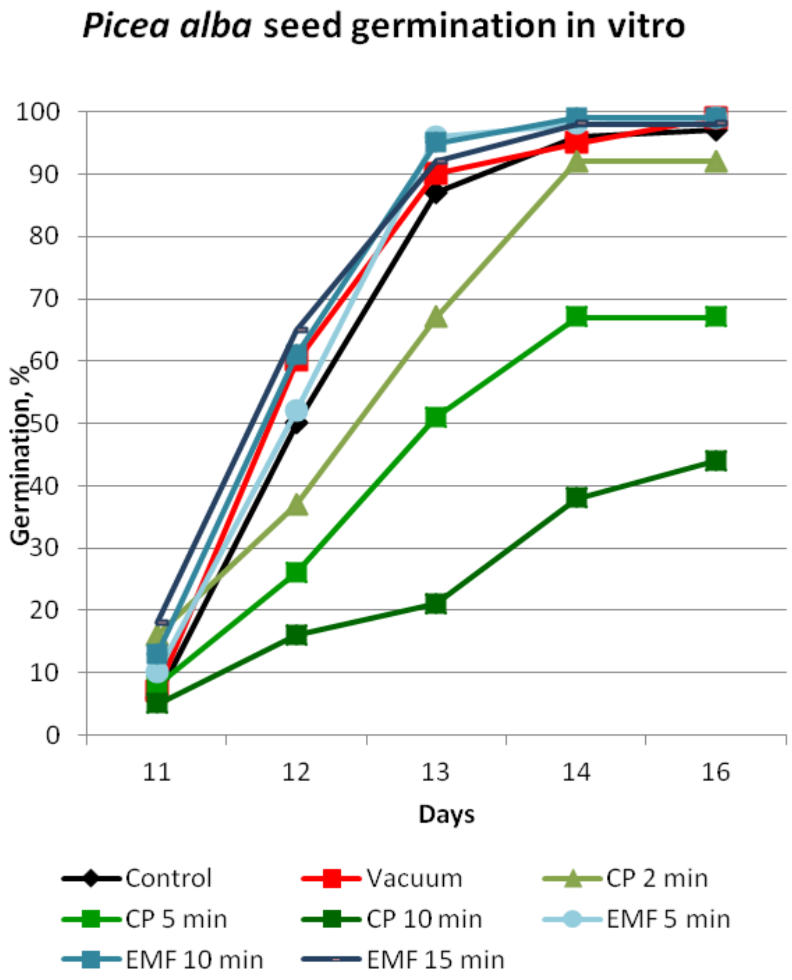
2020

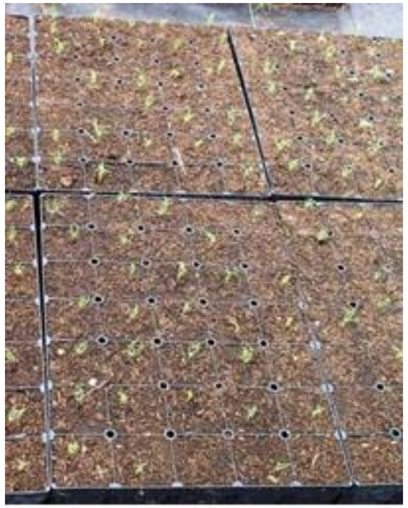
field (forest tree nursery)

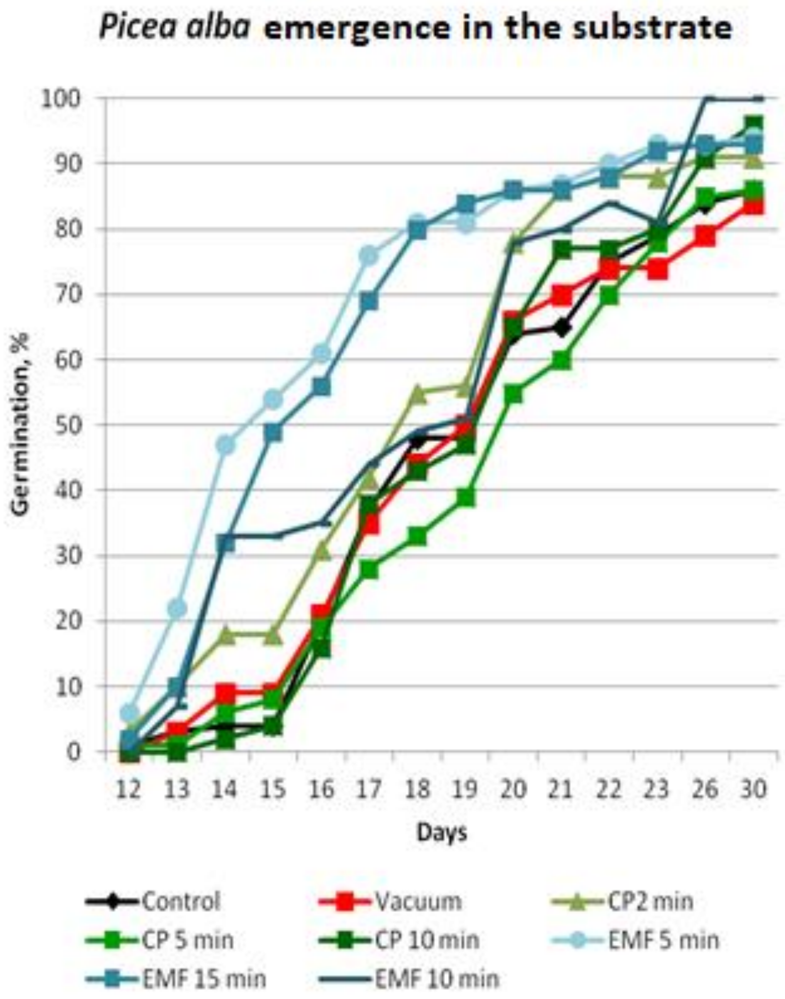


40-50 plants per experimental group.
The total size of collection – over 400 seedlings…

2015: Effects on seed germination and emergence







Growth dynamics of Norway spruce seedlings

Unpublished results removed

in nursery

in pots

outdoors

- 17 month-age seedlings grown from CP (5 min) and CP (7 min) treated seeds, had 50–60% larger height and 40–50% increased branching in comparison to the control.
- In 39-month period after sowing plants grown from vacuum and CP (5 min) treated seeds were higher (by 26 and 17%, respectively) and had larger (by 33 and 21%, respectively) number of branches, as compared to the control.
- After planting to the nursery, growth of seedlings from all EMF groups exceeded the growth of other seedlings so that in 2022 and 2023 only in CP2 and EMF10 groups morphometric parameters were not different from the control, in the rest groups seedlings performed better compared to control. Seedlings in vacuum group still showed the best result

Amount of trichomes on Norway spruce needles and the length of needles in 2017


A. Needle of present year in control group

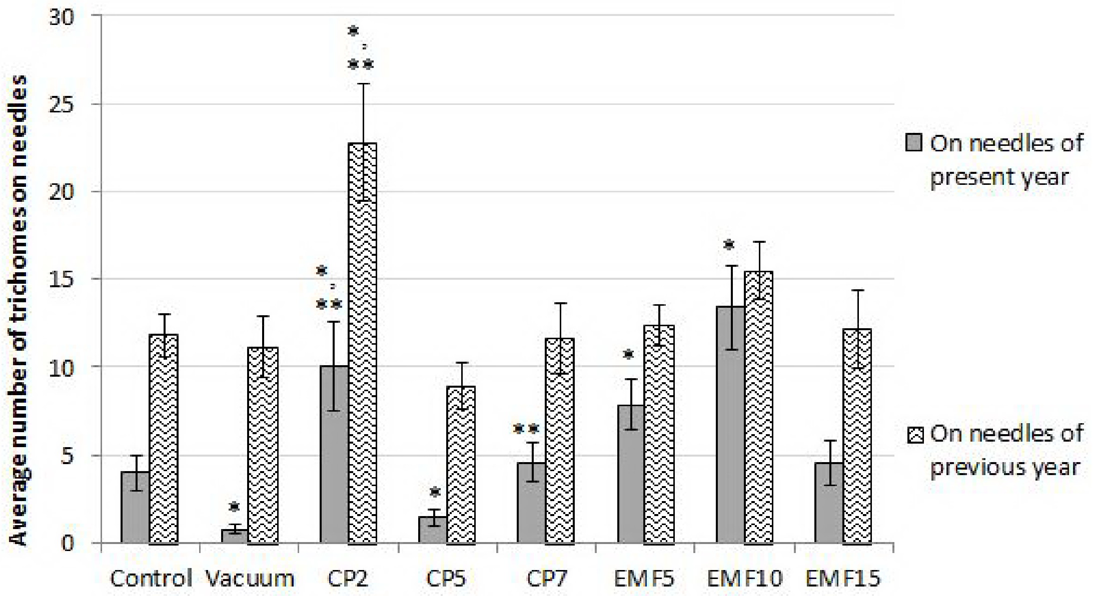

D. Needle of previous year in control group

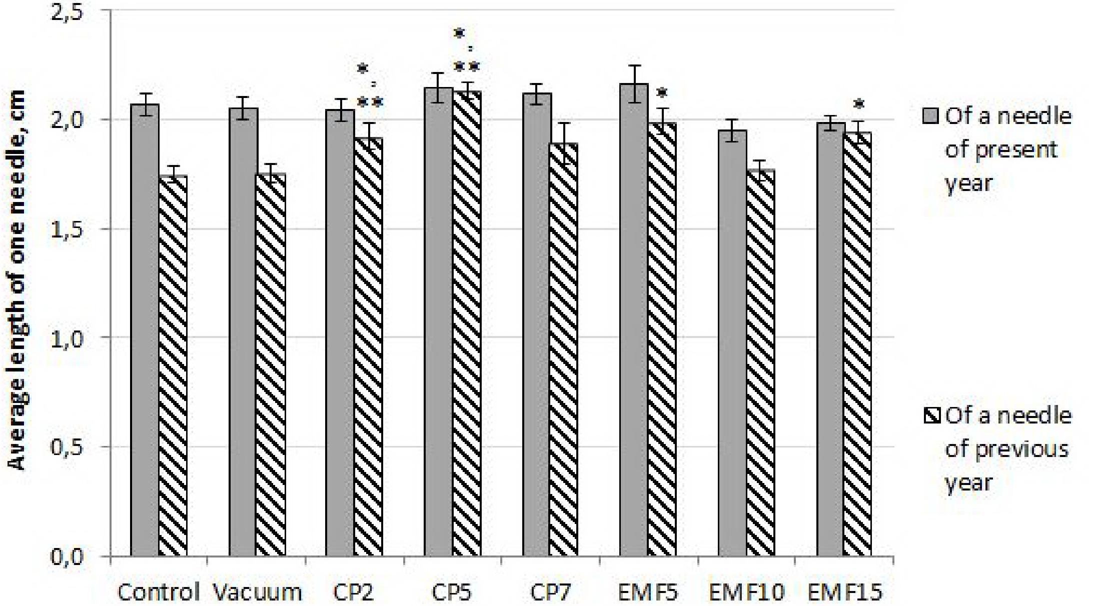

B. Needle of present year in CP2 group


E. Needle of previous year in CP2 group


C. Needle of present year in EMF15 group


F. Needle of previous year in EMF15 group





Amount of secondary metabolites in the needles of Norway spruce in 2017

in collaboration with prof. Ilse Kranner group, Department of Botany, University of Innsbruck, Austria

Analysis performed by liquid chromatography-mass spectrometry.

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CP and EMF treatments increased concentration of shikimic acid and quercetin in mature needles, but the concentration of stilbenes was lower in treated groups as compared to the control.

Differences in the diversity of fungal communities in the mature needles (2017): results of microorganism sequencing

Seed treatments increased fungal diversity: The sequencing revealed 116 different fungal taxa. The highest diversity of fungal taxa was found in samples exposed to EMF10 (77 taxa), lowest – in the control samples (20 taxa).

The most common fungal taxon in all sample types were the same: *Mycosphaerella tassiana* (23.4 %), *Helicodendron coniferarum* (17.2 %), Vishniacozyma sp. 5211_37 (14.7 %), Dioszegia sp. 5211_62 (13.4 %).

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Amount of detected fungal taxa on the obtained fungal sequences in different types of samples.

Venn diagram showing the similarity of fungal taxa in different samples.

Similarity of fungal taxa: there were 9 common fungal species in all samples, of which 7 are the most commonly found among all identified fungal taxa. The highest number of unique (only for that effect in samples) fungal taxa was found in EMF10 exposure samples (30). Among these fungal taxa, there were no plant pathogenic. The least number of unique fungal taxa (2) was present in the control samples: all **treatments promote the emergence and abundance of fungal species diversity.** However, pathogens such as *Ophiosphaerella agrostidis*, *Alternaria alternata*, which were significantly more abundant in the affected needles, were not detected in the control samples.

Fuctional groups of fungi

Relative abundance of the fungal functional groups in samples of the *Picea abies*.
The other functional group are fungi not related to plants and their tissues

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An analysis of fungal functional groups shows that the distribution is similar in all groups.
However, the effect of CP5 was due to a decrease in the abundance of pathogenic fungi, which was mainly due to a half-abundance of *Mycosphaerella tassiana* (15.7 %) than in other sample types.

Conclusion: seed treatments promote the diversity of fungal species, but among the newly emerging species, plant pathogenic organisms also appear.
The question remains whether the effects only create favorable conditions for the emergence of organisms, whether the trees become weaker and their needles are then easily colonized by new fungal species.

Antioxidative activity and content of phenols and flavonoids in the needles of Norway spruce seedlings in 2021 and 2022

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Both in Sept 2021 and Jun 2022, seed treatments resulted in decreased antioxidative activity and amounts of TPC and flavonoids in seedling needles

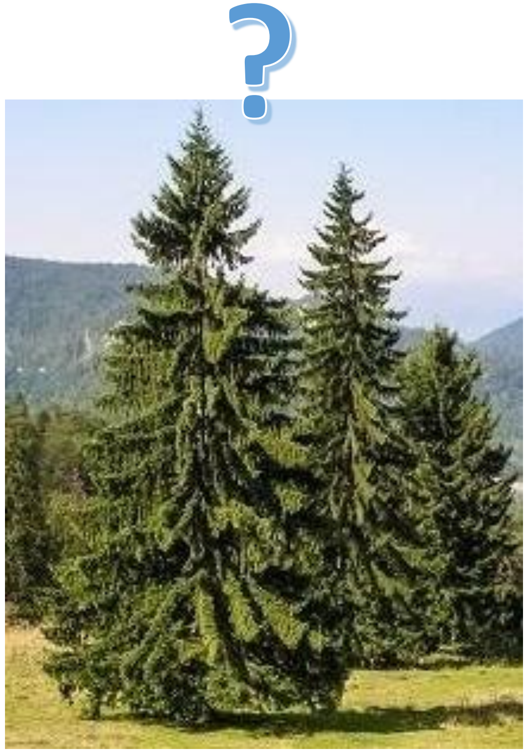
Compared to June 2022, effects in Sep 2021 were much smaller (25-60 % versus 7-27 %)

The largest negative effects were observed in EMF5, CP7 and EMF15 groups; the smallest effects – in vacuum group.

Possible explanation – trade-off effects – the balance in distribution resources between growth and defence?

Conclusions

- The effects of seed treatments with CP (2-7 min), vacuum (7 min), EMF(5-15 min) on growth and biochemical traits of Norway spruce seedings **persist for at least 9 years** (the entire period of observation);
- The dynamics of the observed effects on growth is complex and depends on the conditions of seedling cultivation. Do not expect that one time/condition point shows "a final truth".
- The effects on the secondary metabolism and antioxidative capacity are persistent as well;
- The effects on the composition of the communities of plant associated microorganisms (i.e fungal diversity) are also persistent.



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Thank you for attention